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Characteristics of Hormogonia Formation by Symbiotic *Nostoc* spp. in Response to the Presence of *Anthoceros punctatus* or Its Extracellular Products

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Nostocacean cyanobacteria typically produce gliding filaments termed hormogonia at a low frequency as part of their life cycle. We report here that all *Nostoc* spp. competent in establishing a symbiotic association with the hornwort *Anthoceros punctatus* formed hormogonial filaments at a high frequency in the presence of *A. punctatus*. The hormogonia-inducing activity was produced by *A. punctatus* under nitrogen-limited culture conditions. The hormogonia of the symbiotically competent *Nostoc* spp. were characterized as motile (gliding) filaments lacking heterocysts and with distinctly smaller cells than those of vegetative filaments; the small cells resulted from a continuation of cell division uncoupled from biomass increase. An essentially complete conversion of vegetative filaments to hormogonia occurred within 12 h of exposure of *Nostoc* sp. strain 7801 to *A. punctatus* growth-conditioned medium. Hormogonia formation was accompanied by loss of nitrogen fixation (acetylene reduction) and by decreases in photosynthetic CO_2 fixation and in vivo NH_4^+ assimilation of 30% and approximately 40%, respectively. The rates of acetylene reduction and CO_2 fixation returned to approximately the control rates within 72 to 96 h after hormogonia induction, as the cultures of *Nostoc* sp. strain 7801 differentiated heterocysts and reverted to the vegetative growth state. The relationship between hormogonia formation and symbiotic competence is discussed.

Cyanobacteria often form filaments of distinctly different cellular dimensions and behavioral characteristics relative to the predominant vegetative filaments. Hormogonia are an example of such differentiated filaments; they are generally associated with vegetative reproduction (13). In members of the genus *Nostoc*, hormogonia are defined as filaments that lack heterocysts and have one or more of the following properties: rapid gliding motility, smaller cell size, and different cell shape, all in comparison with vegetative cells and filaments (13). Hormogonium cells often contain gas vacuoles when vegetative cells do not. Formation of hormogonia occurs as part of the nostocacean life cycle (9) and, provisionally, is the characteristic that may distinguish the genera *Nostoc* and *Anabaena* (14). Other than gliding motility and gas vacuole synthesis, the physiological characteristics of hormogonia have not been determined. They were proposed to be growth-precursor cells, analogous to the swarmer cells of prosthecate bacteria (4); that is, a nongrowth state capable of survival under unfavorable environmental conditions. It has also been suggested that the gliding motility of hormogonia serves as a means of dispersal for cyanobacteria living in contact with the substratum in terrestrial or benthic habitats (4). Hormogonia that contain gas vacuoles have the additional advantage of buoyant dispersal in the water column of flooded soils and lentic habitats (1).

Stimulation of hormogonia formation may be induced following changes in a variety of environmental conditions, such as transfer of *Nostoc muscorum* from darkness to red light (650 nm) (8), transfer of *Mastigocladus laminosus* from liquid culture to solid medium (7), transfer of a variety of *Nostoc* spp. from aged to fresh medium (13), and transfer of *Calothrix* sp. strain PCC 7601 from aged to fresh medium under red light (2). In addition to abrupt environmental changes, the nutritional state of the culture can induce hormogonia formation. In *Calothrix parietina* D184, cultures starved for phosphorous form hormogonia (19), as do iron-starved cultures upon addition of iron (3); a culture of *N. muscorum* also produces hormogonia when either deprived of $NaNO_3$ or subjected to an increase in light intensity (1). A thermolabile extracellular metabolite of autogenic origin was found to induce hormogonia formation in dark-incubated cultures of *N. muscorum* and *N. commune*, but the metabolite was not characterized (14).

An apparent correlation between the presence of motile hormogonia and the occurrence of *Nostoc* strains in various symbiotic associations has been noted (6, 15, 17). Establishment of symbiotic associations with hornworts and liverworts depends on infection and colonization by the *Nostoc* spp. of slime cavities on the ventral surface of the gametophyte thallus; the cavities are continuously open to the environment through pores (5). We have observed, and report here, an extensive conversion of free-living *Nostoc* vegetative filaments into hormogonia within the first 24 h of coculture with the hornwort *Anthoceros punctatus* L. The induction of hormogonia appears to be mediated by a soluble compound produced by *A. punctatus* when deprived of combined nitrogen. The physiological characteristics of hormogonial filaments of *Nostoc* sp. strain 7801, a direct isolate from *A. punctatus*, are described in this paper.

MATERIALS AND METHODS

Cultures. Sources and culture conditions for symbiont-free *A. punctatus*, symbiotic *A. punctatus-Nostoc* sp. strain 7801 (penicillin treated to remove epiphytic cyanobacteria), and all free-living *Nostoc* strains were previously described (6).

Response to the presence of *A. punctatus*. Symbiotic competence and extent of hormogonia formation in response to *A. punctatus* were determined by coculture of *Nostoc* spp. and symbiont-free *A. punctatus* gametophyte tissue in the

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medium for culture of *A. punctatus*, lacking combined nitrogen. After 24 h of coculture, a random subsample of the *Nostoc* population was removed and the extent of hormogonia formation was determined by light microscopy. Typically, a scan of an entire wet mount was sufficient for estimation of the fraction of hormogonial filaments in the population. The percentage of hormogonial filaments in the population was estimated and scored as one of four levels: 0% for cultures in which no hormogonial filaments were seen, ca. 10% for low (approximately 5 to 15% hormogonia), ca. 50% for intermediate (40 to 60% hormogonia), and >90% for high (90 to 100% hormogonia) levels. Frequencies of hormogonia formation not included in the categories outlined above were rare and were scored as the closest lower level. Representative photomicrographs of scan views of vegetative and hormogonial filaments are shown in Fig. 1A and B. Symbiotic competence was determined by the presence or absence of *Nostoc* colonies in *A. punctatus* tissue and dinitrogen-dependent growth of *A. punctatus* after 5 weeks of incubation as described previously (6). Basal levels of *A. punctatus*-independent hormogonia formation were determined by using duplicate flasks incubated without *A. punctatus* tissue.

Hormogonia formation and recovery were also monitored by coculture of the two axenic partners separated by 50-kilodalton molecular-mass-cutoff dialysis tubing. Symbiont-free *A. punctatus* tissue was placed within processed and sterilized dialysis tubing, and the tubing ends were clamped. The *A. punctatus* in dialysis tubing was then submerged in a flask containing free-living *Nostoc* sp. strain 7801 in *A. punctatus* medium lacking combined nitrogen and incubated under *A. punctatus* growth conditions (6). A random subsample of the *Nostoc* culture was removed at 24-h intervals to determine chlorophyll *a* (Chl *a*), protein content, in vivo rates of acetylene reduction, $^{14}CO_2$ fixation, and $^{13}NH_4^+$ uptake.

***A. punctatus* growth-conditioned medium.** *A. punctatus* growth-conditioned medium was obtained by transferring 7 to 10 g (fresh weight) of gametophyte tissue (previously maintained in growth medium containing combined nitrogen) to 100 ml of medium lacking combined nitrogen. The culture was incubated at 7 W/m^2 (continuous lighting) and 20°C with shaking for 48 h. The *A. punctatus* tissue was then removed by filtration through sterile Whatman GF/C filter pads. At time zero, *Nostoc* sp. strain 7801 was harvested by centrifugation at 1,000 × *g* for 5 min. The pellet was then suspended in the *A. punctatus* conditioned medium at a density of 1 to 2 μg of Chl *a* per ml. These cultures were incubated as in the coculture experiments. Random subsamples were removed at several time points during the first 12 h to monitor the rate of acetylene reduction. At subsequent 24-h intervals, subsamples were also removed for assays of acetylene reduction and $^{14}CO_2$ fixation.

Nostoc cell numbers were determined by using a hemacytometer and light microscopy at time zero and 24 h after exposure to *A. punctatus* conditioned medium. Because of the gliding motility of hormogonia, subsamples were harvested by centrifugation for 2 min in a microcentrifuge and the pellet was suspended in 50% glycerol for mounting in the hemacytometer. Counts were completed within 20 min of suspension in glycerol.

Activity and biomass measurements. Acetylene reduction assays were done as previously described (6). Rates are reported as nanomoles of ethylene produced per minute (milliunits).

Light-dependent CO_2 fixation by *Nostoc* cultures was measured by assimilation of $^{14}CO_2$. Duplicate aliquots of 1.80 ml of culture were placed in borosilicate glass culture tubes (12 by 75 mm), loosely fitted with plastic covers. After the addition of 0.10 ml of 50 mM $NaHCO_3$ (pH 7) to each tube, the samples were allowed to equilibrate for 5 min at room temperature (23.8 ± 0.8°C) with magnetic stirring (ca. 150 rpm) under direct lateral illumination (17 W/m^2). At time zero, 0.10 ml of 50 mM $NaH^{14}CO_3$ (pH 7) (final specific activity, 0.10 μCi/μmol of bicarbonate; ICN Radiochemicals, Inc., Irvine, Calif.) was added to a final concentration of 0.50 μCi/ml. Samples of 0.35 ml were removed from each tube at 10- to 15-min intervals for up to 1.25 h. These samples were filtered (pore size, 0.45 μm; Millipore Corp., Bedford, Mass.) and rinsed twice with 5 ml of 50 mM $NaHCO_3$ (pH 7). The rinsed filters were placed in 20-ml plastic liquid scintillation vials with 10 ml of liquid scintillation cocktail (Liquiscint; National Diagnostics Inc., Manville, N.J.), and radioactivity was determined with a calibrated liquid scintillation system (LS 7500; Beckman Instruments, Inc., Fullerton, Calif.). Carbon dioxide fixation rates were calculated by using simultaneous equations based on a final specific activity of 0.10 μCi/μmol and linear regression. The rates of darkened controls were subtracted for calculation of light-dependent CO_2 fixation rates. No attempt was made to correct for cellular bicarbonate pools. Rates are reported as micromoles of CO_2 assimilated per minute (units) (Table 3) or as milliunits.

The assimilation of $^{13}NH_4^+$ by *Nostoc* sp. strain 7801 following dialysis coculture with *A. punctatus* was determined as previously described (10).

Chl *a* was extracted from *Nostoc* samples with 90% methanol and quantitated by measurement of A_{665} (11). The pellet from methanolic extraction was suspended in distilled water, disrupted by cavitation for 2 min/ml of suspension (model W225R; Heat Systems, Plainview, N.Y.), and analyzed for protein by the method of Lowry et al. (9) with bovine serum albumin as the standard.

RESULTS

The hormogonia formed by the *Nostoc* spp. studied here are distinguished from vegetative filaments by their shorter filament length, gliding motility, lack of heterocysts, and distinctly differently shaped cells. During hormogonia formation, the cell junctions between heterocysts and adjacent vegetative cells are stretched until the heterocysts are detached from the filaments (Fig. 1E). Hormogonial cells are typically smaller and more elongated than the square cells of vegetative filaments (compare Fig. 1C and D) with tapered end cells. These characteristic end cells eventually differentiate into heterocysts as the filaments return to the vegetative growth state (Fig. 1F).

Induction of hormogonia. The four symbiotically competent *Nostoc* strains examined here formed hormogonia at elevated levels in the immediate presence of *A. punctatus* (Table 1). Two strains originally obtained from symbiotic associations, *Nostoc* sp. strain 7801 (from *A. punctatus* [7]) and *Nostoc* sp. strain 29133 (from the cycad *Macrozamia* sp. [14]), showed the most extensive conversion of vegetative filaments to hormogonia within 24 h of coculture (Fig. 1). Two other symbiotic strains originally isolated as free-living cultures, *Nostoc* sp. strain 27895 (from soil) and *Nostoc* sp. strain 29150 (from a freshwater pond), also converted approximately 50% of their vegetative filaments to hormogonia in the presence of *A. punctatus*. Those *Nostoc* strains surveyed which were not symbiotically competent with *A.*

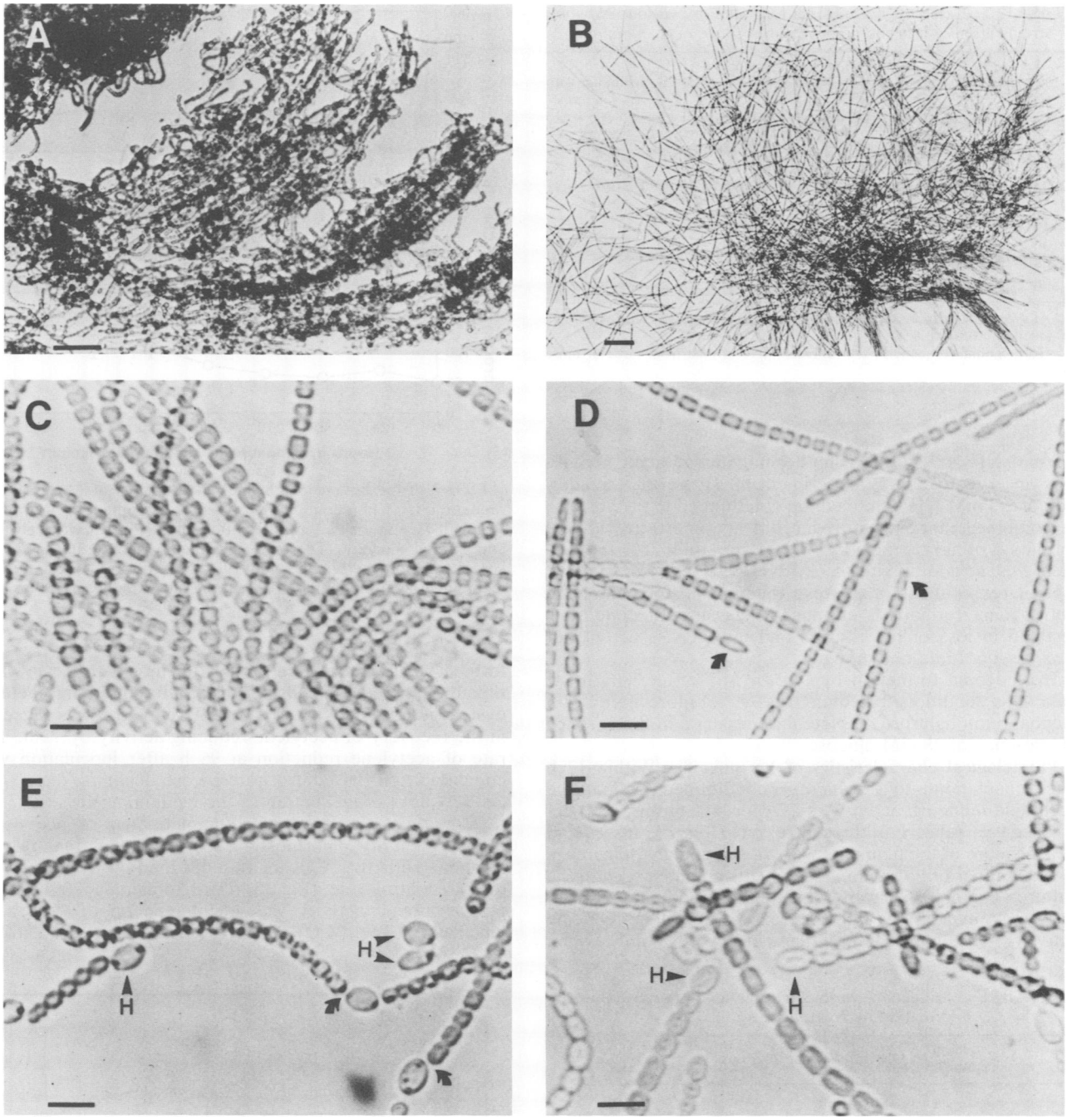

FIG. 1. Bright-field photomicrographs, at low magnification, of *Nostoc* sp. strain 29133, illustrating the overall morphological difference between (A) vegetative filaments and (B) >99% hormogonial filaments. (C to F) Higher magnification of *Nostoc* sp. strain 7801 showing (C) vegetative filaments in contrast to (D) hormogonial filaments (tapered end cells are indicated by arrows). (E) Arrows indicate stretched cell junctions between vegetative cells and heterocysts. (F) At 72 h after coculture with *A. punctatus*; H, heterocyst. Bar markers represent 20 μm in panels A and B and 5 μm in panels C to F.

punctatus failed to form elevated levels of hormogonia in the presence of *A. punctatus*. The single exception was the nonsymbiotic *Nostoc* sp. strain 27896, a pond isolate; in this strain more than 90% of the filaments were converted into hormogonia in the presence of *A. punctatus* (Table 1).

The extent of hormogonia formation in medium conditioned by culture with *A. punctatus* for 24 to 72 h was comparable to that in direct coculture with symbiont-free or reconstituted *A. punctatus-Nostoc* sp. strain 7801 tissue (Table 2, experiments 2, 3, and 4). The pH of conditioned medium was typically lower (pH 5.5 to 6.0) than that of fresh medium (pH 6.4). However, adjusting the pH of conditioned medium did not alter its hormogonia-inducing activity. This result indicates that hormogonia induction in conditioned medium was not the consequence of an abrupt shift in pH.

In medium conditioned by *A. punctatus* in the presence of

TABLE 1. Extent of hormogonia formation by various *Nostoc* strains after 24 h in the presence or absence of *A. punctatus*

Nostoc strain[a]	% of hormogonial filaments[b]		Symbiotic with *A. punctatus*[c]
	A. punctatus absent	*A. punctatus* present	
7801	ca. 10	>90	+
29133	ca. 10	50–90	+
27895	ca. 10	ca. 50	+
29150	ca. 10	ca. 10	+
Mac R1, R2	0	0	−
29106	0	0	−
27904R	0	0	−
27896	0	>90	−

[a] All *Nostoc* strains are capable of N_2 fixation; sources and culture histories are given in reference 6.
[b] Hormogonial frequencies are representative of data from three or more trials.
[c] Symbols: +, symbiotically competent; −, nonsymbiotic with *A. punctatus* (see reference 6).

2.5 mM NH_4NO_3, little or no hormogonia-inducing activity was observed (Table 2, experiment 6). However, the addition of 2.5 mM NH_4^+ to medium conditioned in the absence of combined inorganic nitrogen did not interfere with the formation of hormogonia by *Nostoc* sp. strain 7801 (experiment 8). The addition of 2.5 mM NH_4NO_3 (experiment 5) or NH_4Cl (experiment 7) to fresh medium did not stimulate hormogonia formation, although these growth conditions resulted in loss of heterocysts and nitrogenase activity of *Nostoc* spp. (data not shown).

Hormogonia formation by *Nostoc* sp. strain 7801 in *A. punctatus* conditioned medium was 99% or more after a 24-h incubation in light or complete darkness and in the absence or presence of 28 mM glucose.

Physiological characteristics of hormogonia. Within 12 h after inoculation into *A. punctatus* conditioned medium, the acetylene-reducing activity of *Nostoc* sp. strain 7801 decreased 95% relative to the control rate (Fig. 2A, inset), and 99% or more of the filaments were hormogonia. An increase in the number of hormogonial filaments could be seen in the cultures beginning 4 h after inoculation, and they rose in frequency inversely with the decrease in acetylene-reducing activity. The hormogonial filaments were motile when

TABLE 2. Effects of various treatments on hormogonia formation by *Nostoc* sp. strain 7801

Expt no.	Incubation medium[a]	Additions at time zero[b]	% Hormogonia at 24 h[c]
1	F	None	ca. 10
2	F	*A. punctatus*	>90
3	C	None	>90
4	F	Associated *A. punctatus*-*Nostoc* sp. strain 7801	>90
5	F	2.5 mM NH_4NO_3	ca. 10
6	C[d]	None	ca. 10
7	F	2.5 mM NH_4Cl	ca. 10
8	C	2.5 mM NH_4Cl	>90

[a] In all cases, medium refers to *A. punctatus* growth medium, fresh (F) or conditioned (C) as stated in Materials and Methods.
[b] At time zero, *Nostoc* sp. strain 7801 was suspended in flasks with the respective medium plus supplements. The level of hormogonia in the inoculum was less than 10%.
[c] Hormogonial frequencies are representative of data from three or more trials.
[d] In experiment 6, 2.5 mM NH_4NO_3 was added together with *A. punctatus* when conditioning the medium.

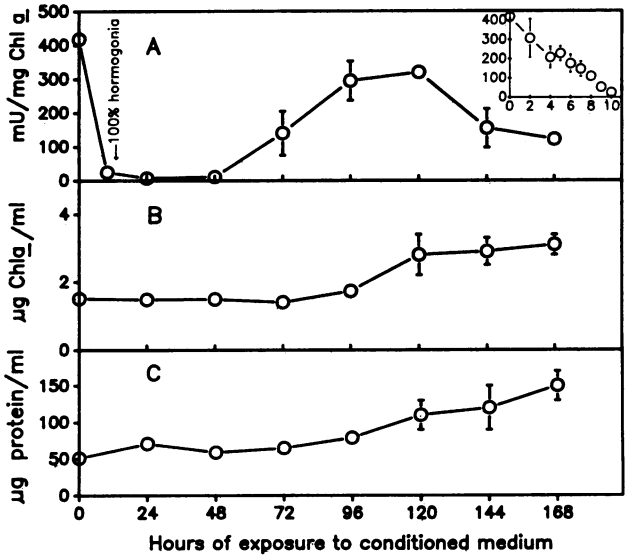

FIG. 2. Time course of changes in the rates of (A) acetylene reduction, (B) Chl *a* content, and (C) protein content of *Nostoc* sp. strain 7801 cultures after inoculation into *A. punctatus* growth-conditioned medium. The inset in panel A shows the decline in specific activity within the first 10 h of exposure to conditioned medium. All datum points represent the mean and standard error of four separate experiments.

formed but ceased gliding 48 h after inoculation, at which time the tapered end cells began to differentiate into heterocysts (Fig. 1F). The heterocyst differentiation was reflected in the recovery of acetylene-reducing activity by 72 h. The rate of acetylene reduction at 96 h after inoculation was comparable to the control rate before declining during the last few days of incubation as the medium aged.

In contrast to the nearly complete loss of acetylene-reducing activity in hormogonia 12 h after induction, the rate of photosynthetic $^{14}CO_2$ fixation declined only 30% relative to the control rate (Fig. 3). The remaining 70% of the photosynthetic activity was maintained up to 72 h after inoculation. The $^{14}CO_2$ fixation rate then increased to ap-

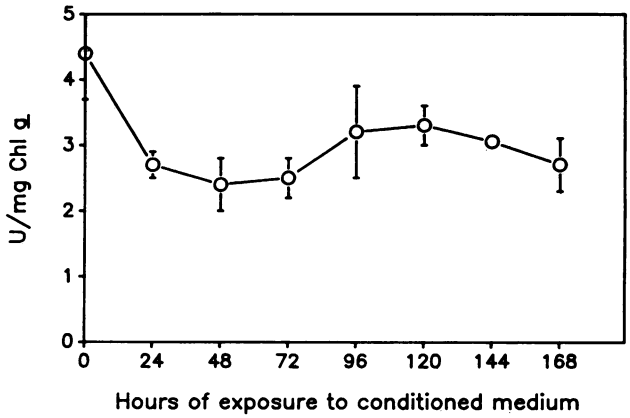

FIG. 3. Time course of changes in the rates of in vivo light-dependent CO_2 fixation by *Nostoc* sp. strain 7801 after inoculation in *A. punctatus* growth-conditioned medium. Datum points represent the mean and standard error of two or three separate experiments.

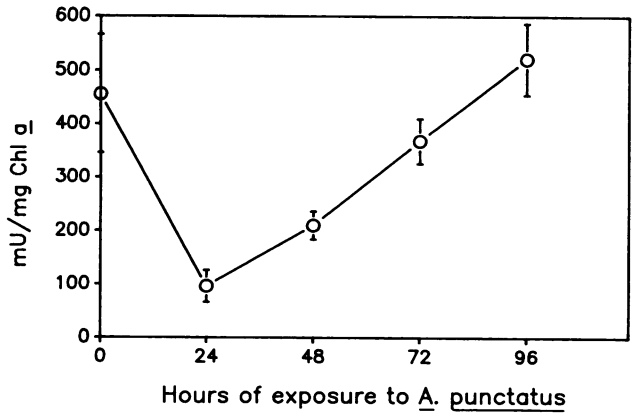

FIG. 4. Time course of changes in the rates of acetylene reduction by *Nostoc* sp. strain 7801 during coculture with *A. punctatus* separated by 50-kilodalton average pore-sized dialysis tubing. Datum points represent the mean and standard error of eight separate experiments.

proximately the same as the control rate by 96 h before declining in a manner similar to the decline in acetylene reduction as the culture medium aged.

In three separate experiments starting with a mean of 2.4 $\times 10^7$ vegetative cells per ml of *Nostoc* sp. strain 7801, there was an increase to a mean of 4×10^7 hormogonial cells per ml (67% increase) within 24 h of exposure to *A. punctatus* growth-conditioned medium. The Chl *a* and protein contents of the *Nostoc* sp. strain 7801 culture, however, did not change markedly during the first 72 to 96 h following induction of hormogonia in conditioned medium, after which both biomass parameters then slowly began to increase (Fig. 2B and C). Slight decreases in Chl *a* content were detected in cocultures of *Nostoc* sp. strain 7801 with *A. punctatus* (data not shown).

In dialysis tubing coculture experiments, hormogonial filaments appeared in the culture beginning at 16 h after inoculation and increased in number until 24 h, when the acetylene-reducing activity reached its minimum. Recovery of acetylene-reducing activity in these experiments, however, was detected within 48 h after the initial exposure to nitrogen-starved *A. punctatus* (Fig. 4). Although the conversion to hormogonial filaments in the presence of *A. punctatus* in dialysis tubing appeared to be complete within 24 h (i.e., no typical vegetative filaments were seen microscopically), 15% of the initial rate of acetylene-reducing activity remained (Fig. 4). The photosynthetic $^{14}CO_2$ fixation and $^{13}NH_4^+$ assimilation activities in hormogonia exposed to nitrogen-starved *A. punctatus* in dialysis tubing were slightly depressed when compared with the activities of vegetative filaments of free-living *Nostoc* sp. strain 7801 (70% and 62% of the control rates, respectively [Table 3]).

DISCUSSION

All 14 *Nostoc* isolates we have tested that will reconstitute in the *A. punctatus* symbiotic association also form motile hormogonia (Table 1) (6; J. C. Meeks, unpublished results). The correlation between symbiotic competence and hormogonia formation (Table 1) implies that the gliding motility of hormogonia is instrumental in colonization by the *Nostoc* spp. of the slime cavities in *A. punctatus* tissue. We suggest that hormogonia serve as the infective units of symbiotically competent *Nostoc* strains and that their formation is the initial stage in establishment of the bryophyte symbiotic association. However, the symbiotic competence of a *Nostoc* strain involves more than formation of gliding hormogonia. For example, *Nostoc* sp. strain 27896 converted more than 90% of its vegetative filaments into hormogonia in the immediate presence of *A. punctatus* or its metabolic products, but this strain repeatedly has not colonized *A. punctatus* tissue within 5 weeks of coculture in medium lacking combined nitrogen, by which time the bryophyte tissue has begun to senesce; in contrast, colonization by *Nostoc* sp. strain 7801 was macroscopically evident within 9 days of coculture.

Several results suggest that the formation of hormogonia by *Nostoc* sp. strain 7801 in response to the presence of *A. punctatus* is mediated by a gametophyte-produced extracellular product(s). First, in all experiments in which hormogonia were induced by transfer of *Nostoc* spp. to medium containing *A. punctatus* or to medium conditioned by culture with *A. punctatus*, no induction occurred in parallel transfers of *Nostoc* spp. to fresh medium (Table 2, experiments 1, 5, and 7). Second, the hormogonia-inducing activity in conditioned medium can be inactivated by autoclaving, which implies the presence of an unstable chemical factor rather than a physical inducer. Third, hormogonia were induced in medium conditioned by culture of *A. punctatus* in the absence, but not the presence, of combined nitrogen; conversely, addition of combined nitrogen to medium conditioned by *A. punctatus* in its absence did not inhibit hormogonia formation. Fourth, hormogonia were formed in conditioned medium under environmental conditions that normally do not induce their formation in other cyanobacteria (i.e., darkness and excess nutrients). The chemical identity of the extracellular hormogonia-inducing activity has not been determined. However, preliminary experiments indicate that the factor(s) has poor long-term storage, is heat labile (by autoclaving), is less than 12 to 14 kilodaltons in size, and is complexed by the addition of polyvinylpyrroli-

TABLE 3. Summary of physiological characteristics of *Nostoc* sp. strain 7801 in free-living vegetative, hormogonial, and symbiotic growth states

Growth state	Doubling time (h)[a,b]	$^{14}CO_2$ fixation (mU/mg of protein)[a]	$^{13}NH_4^+$ assimilation[a,c]
Vegetative	40	102.0 ± 8.6 (n = 15)[d]	13.9 ± 1.9 (n = 6)
Hormogonial[e]		71.4 ± 6.4 (70%) (n = 7)	8.6 ± 2.9 (62%) (n = 8)
Symbiotic	<240 (17%)	16.4 ± 2.8 (16%) (n = 14)[d]	2.9 ± 0.8 (21%) (n = 6)

[a] Values are expressed as the mean ± standard error of n replicates. Numbers in parentheses are percentages of values for the vegetative growth state.
[b] Growth rates are compiled from reference 6.
[c] $^{13}NH_4^+$ assimilation is the amount of ^{13}N recovered in total amino acids as a percentage of $^{13}NH_4^+$ added per milligram of protein. Free-living vegetative and symbiotic values are from reference 10.
[d] These values are from Steinberg and Meeks (in preparation).
[e] Hormogonia were obtained from coculture dialysis tubing experiments as stated in Materials and Methods.

done. We have experimental evidence that neither of the higher plant metabolites luteolin (a flavone that stimulates *nod* gene expression in rhizobia) (12) nor acetosyringone (a phenolic compound that induces *vir* genes in *Agrobacterium tumifaciens*) (16) stimulates hormogonia formation in *Nostoc* sp. strain 7801.

The strong dependence on nitrogen-limited or starved incubation conditions of *A. punctatus* for production of the extracellular hormogonia-inducing activity is compatible with the physiology of the association. When cultured in the absence of combined nitrogen, the symbiotic *Nostoc* colonies within the *A. punctatus* gametophyte tissue differentiate a high frequency of heterocysts and provide newly fixed N_2, as NH_4^+, in amounts sufficient for growth of *A. punctatus* (6). Elevated hormogonia formation in free-living filaments in response to nitrogen-limited or starved *A. punctatus* may serve to increase the frequency and rate of infection by *Nostoc* spp. because the gametophyte tissue requires a source of fixed nitrogen for continued growth. In a similar manner, growth of *A. punctatus* gametophyte thallus from the marginal meristem results in spatial separation between colonies of N_2-fixing *Nostoc* spp. and the regions of highest metabolic activity. The need to colonize the slime cavities in the newly formed gametophyte tissue by hormogonia from *Nostoc* spp. in existing colonies or in the adjacent soil would account for the production of the extracellular hormogonia-inducing activity by N_2-grown associated *A. punctatus*-*Nostoc* sp. strain 7801.

The complete and synchronous conversion of vegetative filaments to hormogonia in *A. punctatus* conditioned medium allowed for physiological characterization of the differentiated filaments. In conditioned medium an increase in hormogonial filaments of *Nostoc* sp. strain 7801 was obvious within 4 h of incubation and reached essentially 100% within 12 h. Since these times reflect less than 25% of the generation time of *Nostoc* sp. strain 7801 under the growth conditions, hormogonia formation is considerably more rapid than formation of heterocysts, which typically requires at least one-half to one generation time (18). The requirement for a longer period for hormogonia formation when *A. punctatus* enclosed in dialysis tubing was added to cultures of *Nostoc* sp. strain 7801 reflects the period necessary for initial production, or accumulation of threshold quantities, of the inducing activity.

The characteristic smaller cells in hormogonial filaments resulted from a continuation of cell division, uncoupled from any increase in biomass. Only after the hormogonial filaments had stopped gliding and begun differentiation of heterocysts did the cultures appear to gain protein and Chl *a*. This response is consistent with the growth-precursor cell concept of hormogonia suggested by Dow et al. (4). Although detachment of heterocysts in the formation of hormogonia in N_2-fixing cultures results in a nitrogen-starved physiology, the mechanism of growth control does not appear to singularly involve inactivation of the carbon- and nitrogen-assimilating systems. The in vivo $^{13}NH_4^+$-assimilating activity and the specific activity of glutamine synthetase (data not shown) of hormogonia were at least 60% of those of vegetative cells. Nevertheless, hormogonia induced in the presence of ammonium also appeared not to increase in biomass during the period of active gliding (data not shown). The rate of in vivo photosynthetic $^{14}CO_2$ fixation by hormogonia, at 70% (Fig. 3; Table 3) relative to the initial rate, also was not substantially depressed. The fate of the photosynthate was not determined.

The reasons why acetylene-reducing activity recovered 24 h earlier when cultures of *Nostoc* sp. strain 7801 were induced in the presence of *A. punctatus* compared with conditioned medium are not clear. It is possible that *A. punctatus* produces a second class of extracellular factors that stimulate heterocyst differentiation and that, owing to instability, are lost in conditioned medium.

The rates of growth (6), photosynthetic CO_2 fixation (N. Steinberg and J. C. Meeks, manuscript in preparation), and NH_4^+ assimilation (10) are depressed approximately four- to fivefold in *Nostoc* sp. strain 7801 when it is in association with *A. punctatus* compared with free-living culture. The cessation of biomass increase and the slight decline in activities of carbon and nitrogen assimilation by hormogonia imply that these filaments may be transitional between the free-living and symbiotic growth states. However, stringent growth regulation and stimulation of heterocyst differentiation apparently do not occur unless the *Nostoc* sp. is within the confines of the cavities in *A. punctatus* tissue.

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